Marked Version of Sequence Listing Amendment

Palatino font is used here, as in the pending application. However, all sequence information in the above "clean version" is in Monaco, 8 pt for allignment purposes.

• *Beginning on page 36, line 18, (ending on page 37) replace the paragraph in the specification with the following;*

The fusion proteins may contain as a heterologous domain a cellular localization domain such as a membrane retention domain. See e.g. PCT/US94/01617, especially pages 26-27. Briefly, a membrane retention domain can be isolated from any convenient membrane-bound protein, whether endogenous to the host cell or not. The membrane retention domain may be a transmembrane retention domain, i.e., an amino acid sequence which extends across the membrane as in the case of cell surface proteins, incluing many receptors. The transmembrane peptide sequence may be extended to span part or all of an extracellular and/or intracellular domain as well. Alternatively, the membrane retention domain may be a lipid membrane retention domain such as a myristoylation or palmitoylation site which permits association with the lipids of the cell surface membrane. Lipid membrane retention domains will usually be added at the 5' end of the coding sequence for N-terminal binding to the membrane and, proximal to the 3' end for C-terminal binding. Peptide sequences involving post-translational processing to provide for lipid membrane binding are described by Carr, et al., PNAS USA (1988) 79, 6128; Aitken, et al., FEBS Lett. (1982) 150, 314; Henderson, et al., PNAS USA (1983) 80, 319; Schulz, et al., Virology (1984), 123, 2131; Dellman, et al., Nature (1985) 314, 374; and reviewed in Ann. Rev. of Biochem. (1988) 57, 69. An amino acid sequence of interest includes the sequence M-G-S S K-S-K-P-K-D-P-S-Q-R [(SEQ ID NO 1)]. Various DNA sequences can be used to encode such sequences in the various fusion proteins of this invention. Other localization domains include organelle-targeting domains and sequences such as-K-D-E-L [(SEQ ID NO 2)] and -H-D-E-L [(SEQ ID NO 3)] which target proteins bearing them to the endoplasmic reticulum, as well as nuclear localization sequences which are particularly useful for fusion proteins designed for (direct) transcription regulation. Various cellular localization sequences and signals are well known in the art.

• *Beginning on page 78, line 28, (ending on page 80, line 6) replace the section in the specification with the following;*

Oligos for construction of calcineurin A fragments:
CNA sequence is in bold

[i]hCNA 5' PCR Oligo Start at residue 12

Junk Xho1
5' cggg ccc ccc ctc gag **tct acg acc gac agg gtg gtg aaa gc** 3' [(SEQ ID NO 4)]

Note: g->t is a silent mutation that destroys the Sal1 site.

[i]hCNA 5' PCR Oligo Start at residue 340

Junk XhoI
5' atat aaa tcg ctc gag **cca tac tgg ctt cca aat ttc atg g** 3' [(SEQ ID NO 5)]

[i]hCNA 5' PCR Oligo Start at Residue 350

Junk XhoI
5' atat aaa tcg ctc gag **ttt act tgg tcc ctt cca ttt gtt ggg g** 3' [(SEQ ID NO 6)]

[i]hCNA 3' PCR Oligo End at Residue 370

Junk See Note ApaI Junk SalI
5' cca gta ggg tct aga tct ggg ccc acg ata taa gtc gac **gtt gag gac**

att tac cag c 3' [(SEQ ID NO 7)]

Note: tct aga tct = overlapping XbaI and BglII sites. [(SEQ ID NO 8)]

[i]hCNA 3' PCR Oligo End at Residue 394

See Note STP FLAG Peptide SalI
5' ttaa tct aga tct tca ctt gtc atc gtc atc ttt ata gtc gac **ctc**

ttt ccg ggc tgc agc tg 3' [(SEQ ID NO 9)]

- *Beginning on page 80, line 9, (ending on page 81, line 3) replace the section in the specification with the following;*

Oligos Designed for Human calcineurin B:
(**Bold** portion is CNB sequence)

[i]hCNB 5' PCR Oligo Start at residue 2

Junk XhoI
5' atat aaa tcg ctc gag **gga aat gag gca agt tat cct ttg g** 3' [(SEQ ID NO 10)]

[i]hCNB 5' PCR Oligo Start at residue 3

Junk XhoI
5' atat aaa tcg ctc gag **aat gag gca agt tat cct ttg g** 3' [(SEQ ID NO 11)]

[i]hCNB 3' PCR Oligo with 3' FLAG peptide and Stop

See Note ApaI STP FLAG Peptide
5' ttaa tct aga tct ggg ccc tca ctt gtc atc gtc atc ttt ata

SalI
gtc gac **cac atc tac cac cat c** 3' [(SEQ ID NO 12)]

Note: tct aga tct = overlapping XbaI and BglII sites. [(SEQ ID NO 8)]

• *Beginning on page 81, line 6, (ending on page 82, line 13) replace the section in the specification with the following:*

Oligos Designed for Constructing CNA-CNB Linkers:
(**Bold** portion is CNA sequence)

[i]3' PCR Primer for hCNA to Generate 24 Amino Acid Template Linker (to residue 370)

Junk ApaI See Note Linker
5' cga ttt atat ggg ccc tct aga tct aga acc aga acc aga acc aga

Linker
acc aga acc aga acc aga acc aga acc aga acc acc aga acc aga acc

acc **gtt gag gac att tac cag c** 3' [(SEQ ID NO 13)]

Note: tct aga tct = overlapping XbaI and BglII sites. [(SEQ ID NO 8)]

[i]3' PCR Primer for Randomizing the Length of the CNA-CNB Linker
(Register 1 oligo)

Junk See Note ApaI Junk SalI
5' g aat cgc aaa tct aga tct ggg ccc gtc atc ttt ata gtc gac acc

aga acc aga acc 3' [(SEQ ID NO 14)]

Note: tct aga tct = overlapping XbaI and BglII sites. [(SEQ ID NO 8)]

[1]3' PCR Primer for Randomizing the Length of the CNA-CNB Linker
(Register 2 oligo)

Junk See Note ApaI Junk SalI
5' g aat cgc aaa tct aga tct ggg ccc gtc atc ttt ata gtc gac aga

acc aga acc aga 3' [(SEQ ID NO 15)]

Note: tct aga tct = overlapping XbaI and BglII sites. [(SEQ ID NO 8)]

- *Beginning on page 84, line 11, (ending on page 85, line 2) replace the section in the specification with the following;*

Generation of the variously lengthed flexible linkers on calcineurin A was accomplished through a two step PCR procedure developed for this purpose. The following bases were added after the codon for residue 370 of calcineurin A by PCR:

CNA residue 370-

GGTGGTTCTGGTTCTGGTGGTTCTGGTTCTGGTTCTGGTTCTGG
TTCTGGTTCTGGTTCTGGTTCTGGTTCT [(SEQ ID NO 16)]

This encodes for the following flexible longest length linker:

GGSGSGGSGSGSGSGSGSGSGS [(SEQ ID NO 17)]
PCR was then performed on the above template with two primers that contained the following complimentary seqence:
Primer 1:
5' GTC GAC AGA ACC AGA ACC AGA 3' [(SEQ ID NO 18)]
Primer 2:
5' GTC GAC ACC AGA ACC AGA ACC 3' [(SEQ ID NO 19)]
and a Sal 1 restriction site (gtc gac) [(SEQ ID NO 20)]. Upon PCR with both primers that can anneal in many registers of the template calcineurin A, fragments of calcineurin A containing from 7 to 24 amino acids of the flexible linker were generated. Interestingly, all of the fragments contained the amino acids GGSGS [(SEQ ID NO 21)] followed by the appropriate number of single alternating Glycines and Serines. The predicted PCR products should have two GGSGS [(SEQ ID NO 21)] repeats, but we recovered only one in all of the clones. Moreover, this strategy also provided fragments that had longer linkers than what we had predicted because the second PCR step allows the linker to grow.

- *Beginning on page 95, line 10, (ending on page 95, line 21) replace the paragraph in the specification with the following;*

To study the ability of the CABs to mediate transcriptional activation in the context of FKBP:FK506, a (XhoI/SpeI) fragment containing the transcriptional activation domain of the p65 subunit of NF-kB was inserted into (SalI/SpeI) digested mCAB constructs. This fusion results in another (SalI/XhoI) fusion which cannot be cut by either enzyme. A similar strategy is possible to generate multimers of the CAB domain, greatly facilitating the production of these reagents. Since all of the restriction enzymes within the coding region are 6-base cutters, they preserve the reading frame for protein synthesis. The mature CAB should have the following amino acid sequence:

NH_2-Met-Leu-Glu-(CnA frag)-Val-Glu-(CnB frag)-Val-Asp-Thr-Ser-COOH

[(SEQ ID NO 22)]

New mCAB-p65 constructs were verified by sequence analysis.

- *Beginning on page 100, line 16, (ending on page 100, line 27) replace the paragraph in the specification with the following;*

The following two oligonucleotides were phosporylated with polynucleotide kinase, annealed, and ligated into pB42AD that had been digested with EcoR1 and Xho 1 to give a new polylinker with the following restriction sites in order.

5′ Xho1-Spacer-Sal1-Nco1-BstEII-BspEI-AflII-ApaI-EcoR1 3′

5′ tcg acg aat tcg ggc ccc tta agt ccg gag gtc acc cat ggg tcg acg tcg gtc gta gac tcg aga 3′

[(SEQ ID NO 23)]

5′ aat ttc tcg agt cta cga ccg acg tcg acc cat ggg tga cct ccg gac tta agg ggc ccg aat tcg 3′

[(SEQ ID NO 24)]

- *Beginning on page 101, line 1, (ending on page 101, line 19) replace the section in the specification with the following;*

The following oligos were used with standard PCR conditions to generate an FKBP fragment with a 5′ EcoR1 and a 3′ BamHI. pLexA and this fragment were digested with EcoR1 and BamH1, gel purified, and ligated.

FKBP oligos:

1. FKBP Y2Hex 5′ (5′ oligo for FKBP with EcoR1 and Xho1 restriction sites

EcoR1 Xho1 Met

5′ c ggg ccc ccc gaa ttc ctc gag atg ggc gtg cag gtg gag ac 3′ [(SEQ ID NO 25)]

2. FKBP Y2Hsb 3′ (3′ oligo for FKBP with 3′Sal 1 and BamH1 restriction sites. No stop codon)

BamH1 Sal1 E
5′ ggg tct gga tcc gtg gac ttc cag ttt tag aag ctc g 3′ [SEQ ID NO 26)]

• *Beginning on page 101, line 21, (ending on page 102, line 6) replace the section in the specification with the following;*

Construction of the miniCABS for pLexA:

mCABS were PCR'd with standard PCR conditions off of my original miniCAB template with the followin oligos and digested with NcoI and BamHI. This gel purified fragment was ligated to gel purified pLexA digested with BamHI and NcoI.

3. mCAB Y2H^{ex} 5′ (5′ oligo for mCAB starting at residue 340 of CNA with 5′ BamH1 and Xho1 sites._

BamH1 Xho1 Pro340
5′ a tat aaa tcg gga tc cgt ctc gag cca tac tgg ctt cca aat ttc atg g 3′ [(SEQ ID NO 27)]

4. mCAB Y2H^{sfann} 3′ (3′ oligo for mCAB with 3′ Sal1, Flag, Stop, Apa1, Not1, and Nco1)

Nco1 Not1 Apa1 stp ----------Flag---------- Sal1
5′ tct ttaa cca tgg cgg ccg c ggg ccc tca ctt gtc atc gtc atc ttt ata gtc gac cac atc tac cac cat c 3′
[(SEQ ID NO 28)]

• *Beginning on page 102, line 27, (ending on page 103, line 17) replace the section in the specification with the following;*

1. Overlap extension mutagenesis of the CNB portion of the mCABS.

5′ oligo for generation of the N-terminal portion of CNB for overlap.

Pflm1
5′ ct tgg tcc ctt cca ttt gtt ggg gaa aaa gtg act gag 3′ [(SEQ ID NO 29)]

3′ oligo for generation of the N-terminal portion of CNB for overlap.

5′ ggg aac aat ctg aaa gat aca cag tta cag c 3′ [(SEQ ID NO 30)]

5′ mutagenic oligo for generation of C-terminal portion of CNB for overlap.

Val119 Met118 Leu115
5′ gctg taa ctg tgt atc ttt cag att gtt ccc (g/c)NN (g/c)NN cat ctt (g/c)NN tac ctg gaa gag ttc ccc 3′ [(SEQ ID NO 31)]

3′ mutagenic oligo for generation of C-terminal portion of CNB for overlap.

XbaI BglII ApaI Stp ---------FLAG--------- SalI 170
5′ ttaa tct aga tct ggg ccc tca ctt gtc atc gtc atc ttt ata gtc gac cac atc tac cac cat c 3′
[(SEQ ID NO 32)]

• *Beginning on page 103, line 19, (ending on page 103, line 30) replace the section in the specification with the following;*

2. Mutagenic oligos for the CNA portion of mCABS.

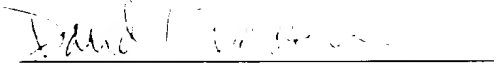
5′ Mutagenic oligo with 5′ Xho I site.

XhoI 340
5′ atat aaa tcg ctc gag cca tac tgg ctt cca aat ttc atg g 3′ [(SEQ ID NO 33)]

3′ Mutagenic oligo

PflM1 353 352
5′ ctc agt cac ttt ttc ccc aac aaa tgg aag (g/c)NN (g/c)NN agt aaa aac atc cat g 3′
[(SEQ ID NO 34)]

Respectfully submitted,



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I hereby certify that this paper is being deposited with the United States Postal Service via First Class Mail under 37 CFR 1.10 on the date indicated above and is addressed to Assistant Commissioner for Patents, Washington, DC 20231

Signed Sue Wilson
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